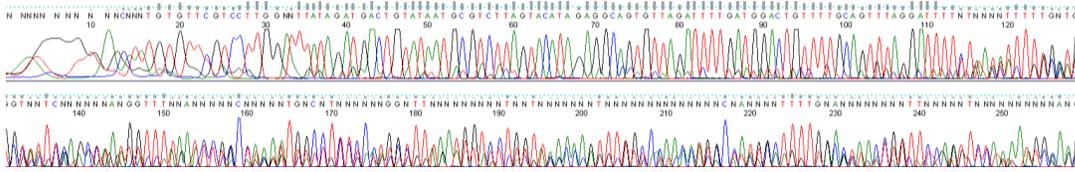# GENEWIZ Solutions Guide: DNA Sequencing
## Non-Specific Results

The following Examples, Possible Causes, and Solutions guidelines will help obtain your best DNA sequencing results. Please email or call GENEWIZ Technical Support for further diagnosis or assistance at dnaseq@genewiz.com or 1-877-436-3949 option 2.


Example 1: Starts as a good signal with a high background, but then becomes non-specific with both peaks becoming equal in height, so the dominant sequence cannot be distinguished from the contaminating sequence.
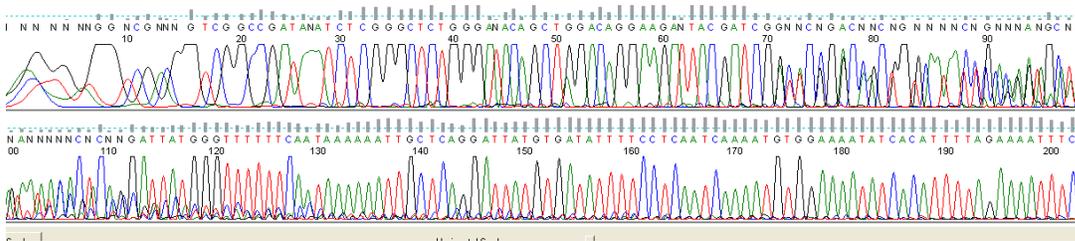

Example 2: Good, clean sequence and then an abrupt change to double peaks (in this case at 71 bp). Also, in this case, the non-specific signal ends at about 130 bp and you see a good, clean sequence again.
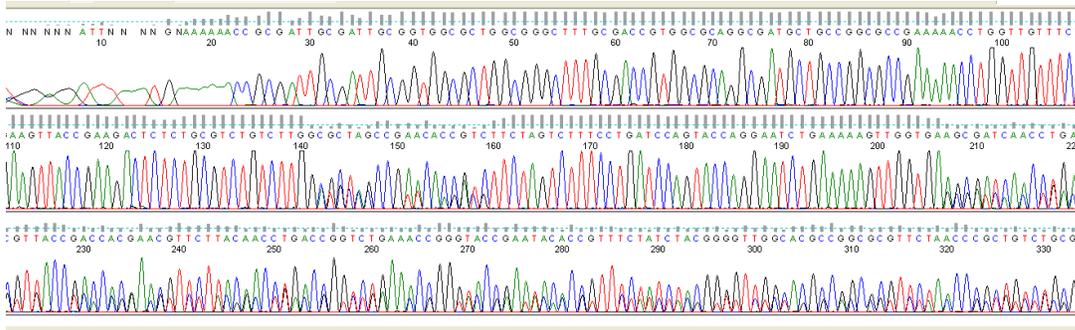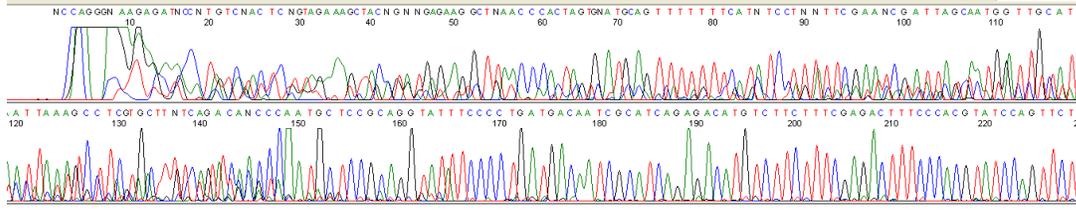

Example 3: Beautiful sequence at the beginning, and then a small patch of non-specific from 142-160 bp before the signal becomes completely non-specific after 208 bp.
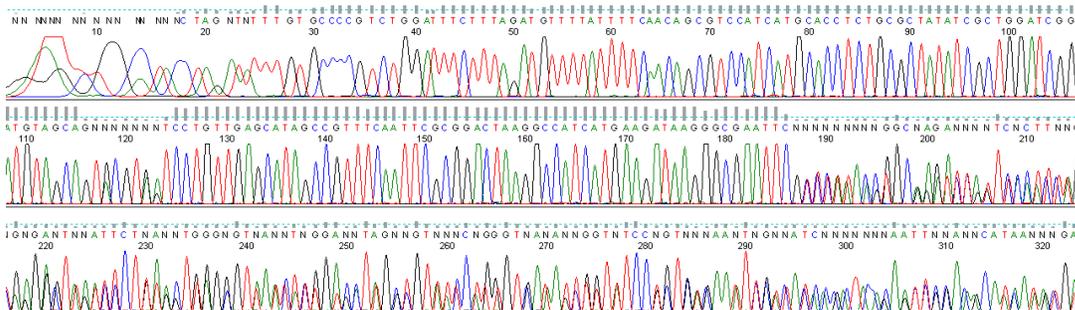
Example 4: Beautiful sequence until 186 bp and then everything is completely non-specific.
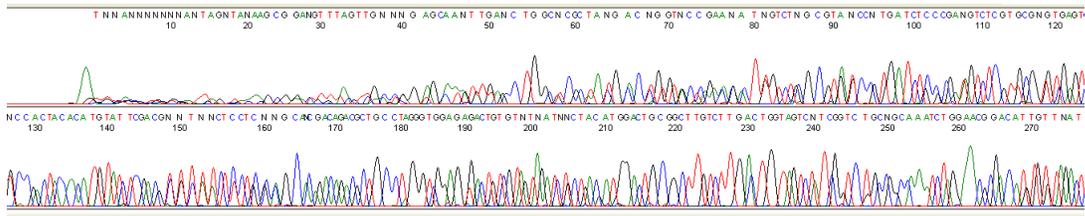
Example 5: Whole sequence is non-specific from the start of the sequence.

Example 6: Signal is non-specific until 150 bp, after which the sequence is beautiful.

Possible Causes:

- Contaminating sequence (Example 1 and Example 5)
- Mixed clone population
  - When using a vector primer, you will see good sequence right up until the insertion site, where you will then see the double or non-specific signal (Example 4)
- Multiple binding sites for the primer on your template
  - Clone from one vector to another and accidentally retain the universal priming site, so now you have two binding sites for the same universal primer.
- Mutation, deletion, or insertion (Example 3)
- Hypervariable region (as found in antibody structures, some mitochondrial DNA, and 16S rDNA) (possible explanation for what is seen in Example 2)

Solutions:

1. If it is a mixed clone population, you will need to re-streak the clone and pick another set of clones to ensure that you have a uniform population.
2. Check your vector and insert for possible secondary priming sites.
3. Try sequencing with a different primer further up- or down-stream from the current primer, or try a primer that will sequence from the other direction.
4. If it is a mutation, deletion, or insertion, hopefully you were expecting it and you are celebrating. If not, you will need to pick a different clone. If it is a true mutation, deletion, or insertion, when you sequence from the other direction you will have good sequence until it encounters the mutation, deletion, or insertion from this direction - as opposed to a mixed clone that will become non-specific at a different point in the forward direction.
5. When cloning from one vector to another, ensure that your restrictions sites do not bracket any of the universal priming sites that may already be on the vector into which you will be cloning.
6. Non-Specific sequence and High Background sequence are sometimes hard to differentiate. We will normally call a sequence non-specific if the contaminating sequence is of equal intensity to the underlying sequence.